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[COMMUNICATION]

Nociception in Crocodiles: Capsaicin Instillation, Formalin and Hot Plate Tests

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ABSTRACT—Three tests of nociception were adapted for the use in crocodiles (47.0–65.2 cm long). In the capsaicin instillation test, capsaicin in concentrations of 10^{-9} to 10^{-3} g/ml instilled in the eye induced concentration related protective reactions which were counted. In the formalin test, 150 μl of 5% formalin was injected subcutaneously in the fore paw, and the time spent "lifting the foot" and "not using the foot" was recorded. In the hot plate test, the plate temperature was set at 55°C and the latency until the following behavioural categories occurred was recorded: "lifting toes", "lifting foot", and "attempt to escape". This test could be repeated with similar results after an interval of 60 min.

It was concluded that the crocodile has a well developed nociceptive system, and it may be possible to study the function of this system using these modifications of well known tests of nociception.

INTRODUCTION

The physiology and anatomy of the nervous system of the crocodile is incompletely known. The embryonic development of crocodilian nervous system has been investigated [1]. Pain perception, and the regulation of pain sensitivity, are basic functions of the central nervous system, with a fundamental biological importance. Knowledge of the physiology of this sensory system and its regulation may be important for the understanding of the physiology and the behaviour of the animal.

MATERIALS AND METHODS

The crocodiles (*Crocodylus niloticus africana*) weighed 231–1125 g, they were 47.0–65.2 cm long, and the abdominal circumference was 9–19 cm. They were estimated to be 4 to 10 months old. The animals were obtained from Mombasa (Kenya) and were transported to Nairobi for experimentation.

The animals were kept in a quiet room in an environment resembling their natural environment. Water was available, as well as stones and sand to lie on. The water temperature was 30.9± 0.2°C and the ambient temperature was 29.5± 3.9°C. The dry surface temperature of the crocodiles was 31.3±0.2°C. Heating bulbs (250 W) were used for maintaining the temperature and evaporating water to maintain the humidity.

The experiments were started a month after the start of the acclimatization. During this period, the animals were handled daily.

Capsaicin instillation

Capsaicin (98%) was supplied by Sigma, U.S.A. A stock solution of capsaicin (1%) was prepared using a vehicle: 10% ethanol, 10% tween 80 and 80% of 0.9% NaCl. Further dilutions were made with 0.9% NaCl.

Capsaicin in concentrations between 10^{-9} g/ml and 10^{-2} g/ml were instilled into the eye according to Gamse *et al.* [2]. Two drops were instilled. The number of protective reactions such as blinking, wiping, blepharospasm, rubbing, head shaking and eyeball movements were counted using a manual counter, during the ensuing period. Blinking and blepharospasm were similar and were therefore scored together. The latency to the first reaction as well as the time-course of the reactions were determined.

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In cotrol experiments, the vehicle was instilled in the contralateral eye. The same eye was used more than once with at least 45 min intervals. Twelve crocodiles were used for the capsaicin experiment. Wire-mesh cages (60×40×27 cm) were used as observation cages.

Formalin test

The formalin test was adapted from that described in rats and mice [3, 4]. A volume of 150 μl 5% formalin was injected subcutaneously in the left fore paw. Two categories of pain-induced behavior were scored: lifing the foot, and completely not using the foot. The crocodiles completely lifted the whole fore leg, from the surface of the observation cages, in the latter behaviour. The time spent in each behavioural category was recorded. Injection of 150 μl 0.9% NaCl in the right fore paw was used as control. Five crocodiles were used. The sequence of saline or formalin injection was random, with an interval of at least 14 days.

The same observation cages were used as in the capsaicin instillation experiment.

Hotplate test

The apparatus used was an IITC Inc Model 35 D Analgesiameter, the temperature was set at 55°C.

Before testing on the hot plate, the animal was placed in a wiremesh cage for 60 min for drying of the skin. Three categories of pain related behaviour were scored: "lifting toes from the plate", "lifting foot", and "attempt to escape". The latency until the behaviour occurred was recorded.

Testing was repeated twice in the same animal with an interval of 60 min and the mean for the three trials was used as the response latency for the animal.

RESULTS

Instillation of capsaicin into the eye

Instillation of capsaicin into the eye produced a number of protective reactions. Blepharospasm was the most common response elicited. At the threshold dose (10^{-9} g/ml) blepharospasm persisted for 3.5±0.5 min (mean±S.E.M). At a concentration of 10^{-3} g/ml, the highest dose studied, the duration of blepharospasm was 18.7±1.0 min. A few trials at a concentration of 10^{-2} g/ml capsaicin resulted in closure of the eye for most of the observation period, and experiments using this concentration were discontinued.

The protective behaviour occurred immediately. There was a distinct dose-response relationship during the first 3 min when capsaicin was instilled at concentrations between 10^{-9} and 10^{-3} g/ml (Fig. 1). In this period the vehicle elicited 2.0±0.3 protective reactions. The threshold concentration (10^{-9} g/ml) produced 5.5±1.0, while the highest concentration (10^{-3} g/ml) used produced 34.7±5.0 protective reactions. Head shaking was observed 6 times: like wiping and eye-ball rolling, head shaking occurred at the highest concentration used (10^{-3} g/ml). These behaviours occurred during the first 2 min. The concentration (10^{-3} g/ml) produced 2.1±0.8 wipings and 10.5±5.2 eyeball movements. The head was raised and vigorously shaken from side to side. The ipsilateral hind paw was raised forwards and used to wipe the eye. Rubbing was scored together with wiping because of their similarity. The eyeball moved up and down its socket during rolling.

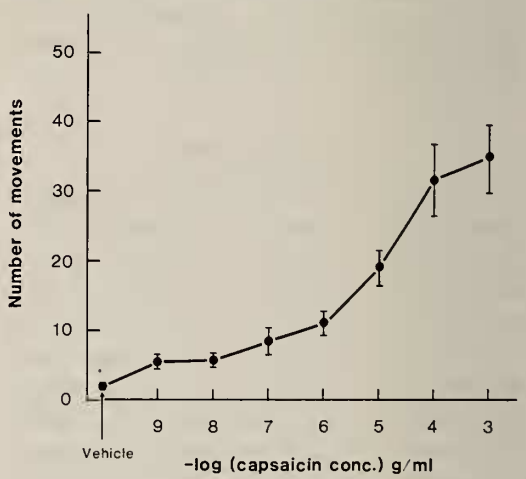

FIG. 1. Number of protective movements in the first 3 min after instillation of capsaicin (10^{-9}–10^{-3} g/ml) or vehicle. Mean±S.E.M. n=13 for each concentration.

For all the concentrations of capsaicin used, most protective reactions occurred during the first 10 min (Fig. 2). No pain behaviour could be observed after the 40 min observation period. At a

concentration of 10^{-3} g/ml, repeated [5] instillation of capsaicin elicited 44.7±4.5, 51.2±6.9, 51.8 ±6.7, 50.3±9.2 and 44.5±5.3 protective reactions, during the first 3 min. No significant change in the response was observed at this and other concentrations used.

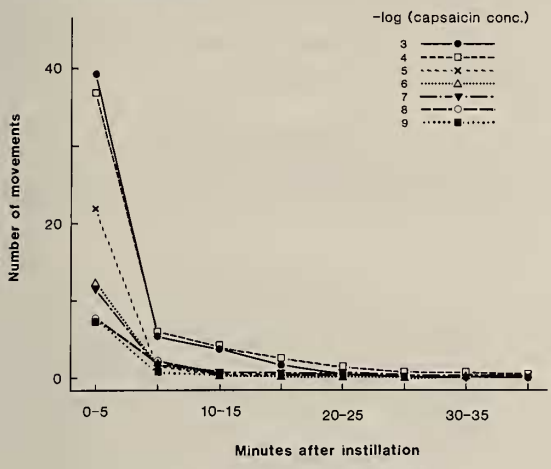

FIG. 2. Time-course of mean number of protective movements after instillation of capsaicin 10^{-9}–10^{-3} g/ml. Blocks of 5 min. n=13 for each concentration.

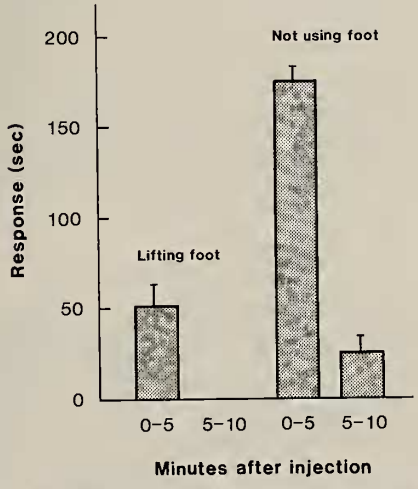

FIG. 3. Time spent "lifting the foot" and "not using the foot" in the formalin test. Blocks of 5 min observation periods. Mean±S.E.M. n=5.

The Formalin test

The formalin injection immediately induced pain related behavioural responses. "Lifting the foot" was observed only in the first 5 min period after injection, while "not using the foot" was observed both in the first and the second 5 min period (Fig. 3). No pain behaviour was observed after 10 min.

Saline injections did not induce this pain behaviour.

The hot plate test

The response latencies for "lifting toes from the plate", "lifting foot", and "attempt to escape" are shown in Figure 4. There were only small and inconsistent differences in the results of the first, the second and the third trial, and no statistically significant difference when the response latencies for the second or the third trial were compared to the first trial ($P<0.05$ for both comparisons for all three latencies, t-test).

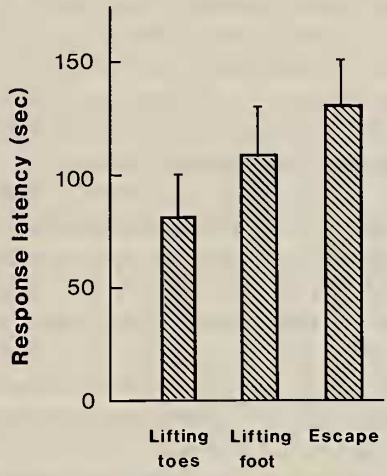

FIG. 4. Response latencies for "lifting toes", "lifting foot" and "attempt to escape" in the hot plate test. Mean±S.E.M. n=5 for animals (n=15 for trials).

DISCUSSION

Our observations indicate that crocodiles are very sensitive to capsaicin unlike amphibians [5] and birds [5–7]. In crocodiles, capsaicin instillation elicited behavioural responses similar to those reported in mammals [5, 8]. The threshold concentration of capsaicin (10^{-9} g/ml) that evoked protective reactions was considerably lower than that (10^{-6} g/ml) evoking responses in guinea pigs and rats [5, 8]. Surprisingly, although crocodiles were sensitive to capsaicin, repeated instillation of capsaicin did not produce any desensitizing effect.

Possibly, capsaicin in crocodiles does not cause a depletion of substance P which may be associated with desensitization [9, 10].

The formalin test in mammals (rat, mice, cat, monkey) elicits both acute and long-lasting pain [3, 4, 11]. The main behavioural responses in these animals are licking, scratching and not using the injected limb. Crocodiles were sensitive to 5% formalin and responded by lifting the foot and not using the foot. The crocodiles did not show a second phase of pain as reported in mammals [3, 4, 11]. The second phase in probably induced by inflammation [3, 4], and requires a stronger stimulus to be elicited than the first phase [Rosland *et al.*, unpubl. data]. The inflammatory stimulus is strong enough to induce licking and scratching in rats and mice, however, it may not be strong enough to induce "lifting the foot" and "not using the foot" in crocodiles.

In the hot plate test, the first response observed in the crocodile was the lifting of one or more toes. It seems that the latency until this response occurs, may be used as a measure of the pain threshold, and may be the response in the crocodile that is the closest to the hind paw lick response in mice and rats [12–15]. The escape response presumably occurs at a stimulus well above pain threshold. The skin temperature may be an important variable in test of nociception applying heat as the stimulus [16]. It is probably important therefore that the ambient temperature is kept constant and that the skin of the feet of the crocodiles is dried well before testing.

All the three tests described here seem to be suitable as tests of nociception in the crocodile. Comparing the three tests, the hot plate test has some advantages as in this test both threshold and suprathreshold responses can be easily and reliably scored, and the test may be repeated even after a rather short interval. We have found that the test is sensitive to morphine and other analgesic drugs [T. I. Kanui *et al.*, unpubl. data]. Since the stimulus as well as the response are different in the three tests, each may be useful for studying different aspects of the regulation of nociception. The tests may also be used to study the influence of drugs on this function of the nervous system in the crocodile.

It may be concluded that the crocodile has a well developed nociceptive system, and it may be possible to study the function of this system using modifications of well known tests of nociception. The nociceptive system is necessary for the survival of the crocodile in the wild. It is responsible for eliciting defense and protective reactions, when crocodiles are attacked by other wild animals or humans.

ACKNOWLEDGMENTS

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